

Birds, Butterflies, and Plant Poisons: A Study in Ecological Chemistry¹

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[For further ecological details of meteorology and biotic zones see "Introduction to the Ecology of the Arima Valley, Trinidad, B.W.I.," by William Beebe, *Zoologica*, 1952, Vol. 37, No. 13, pp. 157-184].

I. INTRODUCTION

IN 1862, Bates proposed the theory of mimicry which included the idea that certain sexually mature insects are brightly colored and behave conspicuously in order to advertise to vertebrate predators the fact that they are unpalatable. Wallace (1867a) extended this to include gaudily colored caterpillars and the experiments of Weir (1869, 1870) and Butler (1869) led to the establishment of the theory of warning coloration (Wallace, 1869, 1870; Darwin, 1871; Poulton, 1887). Slater (1877) first suggested that the unpalatability of warningly colored insects is derived from their larval foodplants, stating that "strikingly colored insects, not otherwise specially protected, will be found to feed upon poisonous plants or upon such as, though not poisonous, possess unpleasant, or at least very powerful odours or flavours" (p. 205). This was accepted by Meldola (1877), and considered probable but

not exclusive by Wallace (1889). Seven years later, Hasse (1896) produced his monumental study of mimicry in butterflies which, along with the works already mentioned, firmly established the hypotheses that (1) species of butterflies differ in their palatability to vertebrate predators, (2) the palatability is correlated with their taxonomic groupings, and (3) those groups which are distasteful feed as larvae upon poisonous plants.

According to the early findings, there are five major groups of unpalatable butterflies. These are (see also Table 1): (1) the tribe Troidini of the subfamily Papilioninae, known as the "pharmacophagus" swallowtails which feed on *Aristolochia* and related plants of the family Aristolochiaceae (Asaraceae). Both the butterflies and the plants are abundantly represented in the New and Old World tropics (excluding Africa) and a few of each extend into temperate regions. (The geographic distribution of the butterflies is based on Seitz, 1924, and that of the plants on Good, 1947). Within the large family Nymphalidae there are four distasteful taxa. Two closely related subfamilies, (2) the Ithomiinae and (3) the Danainae, are both reputed to be unpalatable. The Ithomiinae are almost exclusively neotropical and feed mainly on the Solanaceae. These plants have a distribution similar to that of the Aristolochiaceae. The Danainae are pantropical, but best developed in Africa and the Indo-Australian region, and feed predominantly upon Asclepiadaceae and the related Apocyanaceae, both of which are pantropical, with one genus, *Asclepias*, well developed in North America. The remaining two subfamilies of distasteful species are (4) the Acraeinae and (5) the Heliconiinae. Both feed upon Passifloraceae, another pantropical plant family, but the Acraeinae have been reported from other plants as well (Hasse, 1896; Fawcett, 1901, 1903;

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Wynter-Blyth, 1957). The heliconiines are exclusively neotropical with a few species reaching the subtropics, while the acraeines are found predominantly in the Old World tropics.

The Pieridae were not studied in the experiments presented in this paper, but it appears that some are palatable or Batesian mimics, others are unpalatable and serve either as models or enter into Müllerian mimicry complexes, while still others seem of intermediate acceptability. Thus Wallace (1867b) believed the Indo-Australian pierine genus *Delias* (*Thyca*) to be unpalatable due to the coloration and conspicuous behavior of the butterflies and because certain of the species appear to be mimicked by other, presumably palatable, pierines. Finn (1895, 1897a and b) experimentally demonstrated that *Delias eucharis* Drury was unacceptable to a variety of vertebrate predators. Similarly, the African genus *Mylothris* is also unpalatable, as Swynnerton (1919) established. The neotropical genus *Pereute*, which is very closely allied to *Delias*, (Klots, 1933), is probably also unpalatable and the species may be Müllerian mimics of some of the heliconiines, as Dixey (1894) first suggested. Similarly, the fascinating South American genus *Archonias* (*Euterpe*), now revised to include the former genus *Charonias*, (Klots, 1933), seems to enter a Müllerian complex with both the *Aristolochia* swallowtails and the "tiger-stripe" complex of yellow, black and orange ithomiines, heliconiines and lycoreines. One wonders if Bates' classical *Leptalis* (*Dismorphia*) species upon which his theory of mimicry was based are also unpalatable, as Beddard (1892) suggested. However, the fact that they belong to the most primitive subfamily, *Dismorphiinae*, makes this unlikely for the reasons discussed in Section III-D-5. Further indication of the extreme variability in this family is seen even within a single species: for example, the larvae of *Pieris brassicae* Linnaeus appear unpalatable, whereas the adults are relatively acceptable (Weismann, 1882; Pocock, 1911). On these lines of evidence, and also on the basis of the feeding experiments of Marshall (1902), Carpenter (1921), Finn (1896) and Frazer & Rothschild (1960), it seems best to state provisionally that the Pieridae are intermediate between the five unpalatable groups enumerated above and the palatable Papilionini, Satyrinae, Nymphalinae and Lycaenidae.

Too little is known of the remaining groups in Table 1 to make valid statements concerning their relative acceptability. However, on the basis of both coloration and behavior, it is likely that the Parnassiinae are unpalatable, while the Morphinae and Libytheidae are palatable.

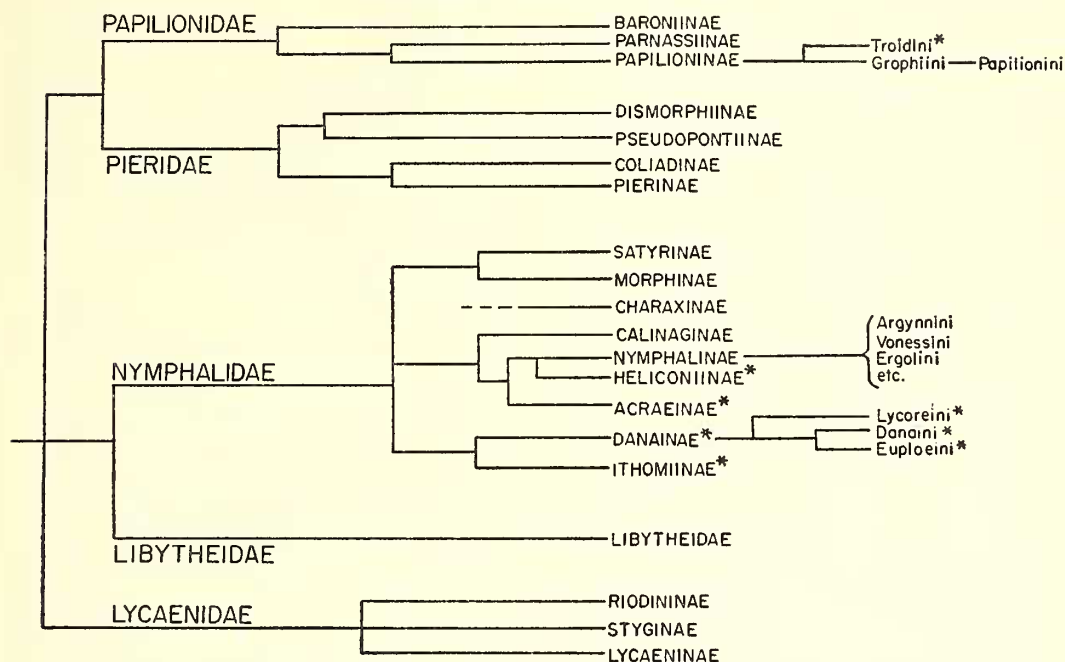
Finally, it is well to remember that exceptions to this general picture will undoubtedly be found. Thus, for example, the nymphaline genus *Adelpha* and the riodinine genus *Nymphidium* have species which mimic each other in the neotropics (Seitz, 1924) and yet both belong to groups considered palatable. Another peculiar nymphaline genus is *Agrias*. These butterflies are among the most gaudily colored members of the animal kingdom and possess complex scent organs. Both of these facts argue that they should be an unpalatable group involved in Müllerian mimicry (L. Brower, 1963), and yet their behavior, typical of cryptic species (Seitz, 1924), supports the classical contention that they are palatable.

While these considerations are no doubt generally correct, it must be emphasized that nearly all the evidence has been obtained from anecdotal accounts or from experiments conducted without adequate controls. As a result, it has not been possible to make quantitative statements regarding the comparative palatability of the numerous species studied. Yet this is a situation greatly to be desired both to provide a solid groundwork for biochemical investigation of the unpalatability of insects in relation to the plants which they eat, and to gain a basis for understanding the evolutionary relationships between the different groups of butterflies as seen in mimicry.

It is towards these ends that the present paper is aimed. In it we shall (1) describe the results of an experiment which quantitatively demonstrates that the groups of butterflies classically held to be unpalatable are in fact so. Following this there will be (2) a discussion of the evolution of unpalatability and the bearing of the experimental findings on Batesian and Müllerian mimicry. Then, (3) by a survey of the phytochemical literature, we shall establish that the groups of plants which are eaten by the larvae of the unpalatable butterflies contain extremely poisonous substances. Finally, we shall attempt to support the thesis that (4) phytophagous insects in the course of their evolution have developed the ability to incorporate poisonous and repellent plant chemicals into their tissues with the result that they are unpalatable to vertebrate predators. The paper will not discuss how a mutation allowing unpalatability to develop can be of advantage to an individual organism prior to its establishment in the population. This genetical problem was originally considered by Eltringham (1909), and Fisher (1958) suggested a plausible solution which Huheey (1961) has recently elaborated.

TABLE 1. PROBABLE RELATIONSHIPS OF THE FAMILIES, SUBFAMILIES AND SOME TRIBES OF THE BUTTERFLIES. BASIC SCHEME MODIFIED FROM EHRLICH (1958). SEE ALSO REFERENCES IN TABLE 2.

* = CLASSICAL UNPALATABLE GROUPS.



II. ACKNOWLEDGMENTS

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III. THE EXPERIMENT

(A). Materials and Methods

From October through December, 1962, six Blue Jays, *Cyanocitta cristata bromia* Oberholser, were trapped in mist nets at Amherst, Massachusetts, and maintained in an aviary for several weeks. They were then transported in a cage to Trinidad, W. I., as hand luggage over a period of 30 hours by air and car. The cage consisted of six individual units 8 inches high by 6 inches wide by 12 inches long, each with a wire screening front, a perch, and with food and water. Upon arrival, the birds were placed in an outdoor aviary in individual experimental cages similar to those at Amherst and described in detail elsewhere (Brower, Brower & Collins, 1963).

From January, 1963, until the experiments were completed, the birds were fed a standard diet of broiler chicken mash mixed with crushed yolks of hard boiled eggs, fine-chopped liver, heart or beef, crushed banana and a small amount of water. This was supplemented by sunflower seeds and paw-paw. A tube of water was kept in each cage and the sand-covered floor was kept clean by periodic sifting. The sex of the jays was not determined.

The ten species of butterflies studied are listed in Table 2, along with their known or suspected larval foodplants. In order to eliminate the variables associated with the two sexes, which include the color-pattern, odor and scent organs, only one sex was offered to the birds. The male was chosen, since females are less easily obtained in the wild. All the butterflies were caught in various parts of Trinidad during the summer of 1963, and only specimens in good condition were offered to the birds. The species were intact, except *Lycorea ceres* from which the scent-disseminating hairpencils had been removed for other purposes. As F. Müller (1878) originally suggested for the lycoreine genus *Ituna*, it is possible that these hairpencils are defensive as well as sexual organs, because they are extruded when the male is held. This is apparently also true of the Euploeini (Varley, pers. comm.), but is not true of *Danaus* (Brower & Cranston, in press). Whether the hairpencils of *Lycorea* enhance its unpalatability is not yet known, and the conclusions in this paper are made on the assumption that they do not.

All species were killed by freezing, spread horizontally, and stored in this position in glassine envelopes (Ward's Natural Science Establishment, Rochester, New York) for two days to two weeks in a deep-freezer (see Brower, Brower & Collins, 1963, for details of preparation). The butterflies were thawed a few minutes before use. This freezing technique greatly facilitates the experimental work, but one possible drawback, as mentioned in our previous paper, is that acetylcholine is inactivated. Fowler & Lewis (1958) have shown that this is due to the action of cholinesterase, which is released when the tissue thaws. Acetylcholine is one of the pharmacologically active substances frequently associated with venoms, venomous tissues or stinging organs in both plants and animals, and probably enhances the effects of the poisons on the victims or increases the responsiveness of their tissues to the noxious substances (Frazer & Rothschild, 1960; Bisset, Frazer, Rothschild & Schachter, 1960). Whether it plays any role in the unpalatability of the butterflies studied is not yet known, but the possibility should be kept in mind.

TABLE 2. CLASSIFICATION OF THE 10 SPECIES OF BUTTERFLIES OFFERED TO THE BLUE JAYS, AND THEIR FOODPLANTS*

Species ¹	BUTTERFLIES			FOODPLANTS		
	Family	Subfamily	Tribe	Species	Family	
<i>Parides neophilus parianus</i> (Hübner)	Papilionidae	Papilioninae	Troidini ²	<i>Aristolochia</i> spp. ⁷	Aristolochiaceae	
<i>Euptychia</i> spp.	Nymphalidae	Satyrinae	Satyrini ^{3, 4}	Various species ^{5, 11}	Graminae	
<i>Heliconius sara thamar</i> Hübner	Nymphalidae	Heliconinae	Heliconiini ^{4, 5}	<i>Passiflora auriculata</i> ⁸	Passifloraceae	
<i>Agraulis vanillae vanillae</i> (L.)	Nymphalidae	Heliconinae	Heliconiini ^{4, 5}	<i>Passiflora foetida</i> ⁸	Passifloraceae	
<i>Biblis hyperia</i> (Cramer)	Nymphalidae	Nymphalinae	Ergolini ⁵	<i>Tragia volubilis</i> ^{9, 11}	Euphorbiaceae	
<i>Anartia amalthea amalthea</i> (L.)	Nymphalidae	Nymphalinae	Vanessini ⁵	Various species ^{5, 11}	Acanthaceae	
<i>Victorina steneles steneles</i> (L.)	Nymphalidae	Nymphalinae	Vanessini ⁵	<i>Blechnum browni</i> ^{9, 12}	Acanthaceae	
<i>Danaus plexippus megalippe</i> (Hübner)	Nymphalidae	Danainae	Danaini ⁶	<i>Asclepias curassavica</i> ^{7, 11}	Asclepiadaceae	
<i>Lycorea ceres ceres</i> (Cramer)	Nymphalidae	Danainae	Lycoreini ⁶	<i>Gonolobus broadwayi</i> ⁷	Asclepiadaceae	
<i>Itomia drymo pellucida</i> Weymer	Nymphalidae	Itomiinae	Itomiini ⁴	Various species ^{7, 10}	Solanaceae	

*The bases for this classification and the larval foodplant records are:

¹All subspecific names from Crane (1954) except *H. sara thamar*, which is from Alexander (1961); ²Munroe (1960) and Munroe & Ehrlich (1951); ³Ehrlich (1958); ⁴Seitz (1924); ⁵Forbes (1939); ⁶present paper, see text; ⁷Alexander (1961); ⁸Kaye (1921); ⁹probable, from ⁹ and ¹¹; ¹⁰W. Müller (1886); ¹¹W. Müller (1886) gives *Stephanophysum longifolium* and other Acanthaceae for the Brazilian *Victorina trayga* Hbn.

The experiment was conducted between 8 a.m. and 12 noon for ten days from August 6 to 15, 1963, inclusive. Food was removed from the birds' cages at 8 a.m. and after about two hours of deprivation they were given the butterflies. In order to place the butterflies in each bird cage without excessively disturbing the occupant, the tray floor was pulled out 12 inches and they were placed dorsal side up on the sand with 12-inch forceps. Because the movements of the birds tended to disturb the arrangement of the butterflies, a pinch of sand was placed on the apices of the fore and hind wings of each. This was done without obscuring their color-patterns. Each tray was then pushed back into position. After a two-hour period, the trays were pulled out and the condition and position of the ten butterflies were noted in detail. All remains of wings and bodies and all intact individuals were removed and tabulated for scoring purposes as in the earlier experiments (not touched, pecked, killed or eaten, J. Brower, 1958a). To avoid confusion it should be emphasized that killed means that a bird mutilated the frozen, dead butterfly to an extent that, had it been alive, it would have died or been unable to reproduce. A dish of fresh food was returned to each cage immediately after the removal of the butterflies at the end of a day's experiment.

(B). Experimental Design

This experiment was designed to compare the palatability of ten species of butterflies to six birds as experimental predators. Five of these butterflies were from groups reputed to be unpalatable, whereas the other five were from groups assumed to be edible. At 24-hour intervals, each bird was presented with an array of 10 butterflies, one each of the 10 species, and was allowed two hours to respond to them. This was repeated for 10 consecutive days so that each bird received a total of 100 butterflies. The positions of the butterflies with respect to each other were the same for all birds on any one day, but were varied at random from day to day. This was done as follows: the butterflies were placed on the floor of each cage in three rows, one with four individuals, and two with three. All butterflies were about one inch apart. Each species was assigned a permanent number from 1 to 10. Slips of paper with these numbers were drawn at random. The butterfly represented by the first number to be drawn was placed in the back row on the left, the second in the same row to the right of the first, and so on, until four had been set out. Then three more were placed in a middle row, and the remaining three in the front row, the last being placed in the right-hand position. In this way, a trapezoid of approximately equi-

distant butterflies was set out, with its base towards the back of each cage.

A criterion for qualification of the predators used in all of our mimicry experiments has been that they must eat at least one of the insect species offered. On this basis, one Blue Jay in this experiment was disqualified because it failed to eat any of the 10 species of butterflies (Table 3).

It is important to emphasize that the birds were caught as adults in North America and shipped to the neotropics; this assured that they could never have had prior experience with any of the butterflies. Moreover, with the exception of the satyrines, *Euptychia* spp., and the danaine, *Danaus plexippus megalippe*, all of the butterflies were of a radically different color-pattern from anything the birds could have seen previously. This is an important difference between this investigation and our earlier experimental studies of mimicry where prior experience with the insects was either indicated or at least a possibility. Another way of obtaining naive predators would have been to hand-rear native birds under a carefully controlled food regimen. But this is costly in time and effort, and was not feasible. Moreover, hand-reared birds may become juvenilized, that is, excessively dependent upon the experimenter, the feeding apparatus, or even the example of another bird, in getting food, and thereby may require an abnormally long time to learn to reject items found to be unpalatable (Swynnerton, 1915, in Carpenter, 1942; Rand, 1942).

There are several other differences from the earlier work. The butterflies were presented simultaneously instead of sequentially, one at a time. Previously, insects of only two appearances were offered to the predators: that of the edible, and that of the model and mimic. In this experiment, the 10 species all differed from each other in size, shape and color-pattern (see black and white illustrations in Crane, 1954; colored illustrations in Seitz, 1924). Again in the earlier work, the experimental and control predators had only two possible differences of palatability to cope with during the learning period; here, there were ten possibilities. Instead of repeated presentations of short duration (1½–2 minutes), the jays in this experiment were allowed two hours to examine and respond to the butterflies. Finally, the total number of presentations of each butterfly species was 10 in this experiment, while in the earlier ones it was generally more. The main consequence of these differences in design is that the birds in the present experiment could not be expected to learn to avoid the butterflies on sight alone. The number of the three most unpalatable species which the

TABLE 4. RELATIVE FREQUENCIES, MEANS, VARIANCES AND TRANSFORMED VARIANCES* OF 10 SPECIES OF NEOTROPICAL BUTTERFLIES NOT EATEN BY 5 BLUE JAYS (Data from Table 3; for statistical analysis, see Table 5)

BUTTERFLIES										
Bird No.	<i>Parides</i>	<i>Danaus</i>	<i>Ithomia</i>	<i>Heliconius sara</i>	<i>Lycorea</i>	<i>Biblis</i>	<i>Euptychia</i>	<i>Agraulis</i>	<i>Anartia</i>	<i>Victorina</i>
2	1.00	.90	.60	.50	.10	.30	.00	.00	.10	.20
3	1.00	1.00	.80	.40	.70	.10	.10	.00	.00	.00
4	1.00	1.00	.90	.90	.70	.20	.00	.20	.10	.10
5	1.00	1.00	.70	.40	.60	.10	.10	.30	.00	.00
6	1.00	1.00	1.00	.90	.00	.30	.40	.10	.30	.10
Means	1.00	.98	.80	.62	.42	.20	.12	.12	.10	.08
Variances	.000	.003	.025	.068	.118	.010	.027	.017	.015	.007
Transformed Variances*	.00	66.3	230.8	282.5	671.3	54.0	265.3	231.3	200.0	145.5
	UNPALATABLE			INTERMEDIATE		PALATABLE				

*See text.

the birds was the same for all species. However, in a study of this kind, it is not always possible to obtain the data exactly as planned; if one bird had died on the ninth day, it would have necessitated converting the data to proportions. Some of the predators died or stopped eating in our previous investigation (Brower, Brower & Collins, 1963) and probably will in future ones. Consequently, for consistency with our past and anticipated work, as well as to facilitate direct comparison, the analysis seemed best carried out on the data in the form of proportions.

1. Relative Palatability of the Ten Species

An examination of Table 4 shows that *Parides* was uniformly not eaten, while all the other species were variably treated. Bartlett's test for the homogeneity of the 10 variances shows lack thereof ($P < .001$, Table 5a). When *Parides* is excluded, and the remaining nine are compared, there is no significant difference in their variances as a group ($.50 > P > .25$, Table 5a). It is thus necessary to consider *Parides* separately from the rest of the species in the statistical analysis.

The *F* test applied to the remaining nine species indicates that the differences of their means are highly significant ($P < .001$, Table 5b). The components of variance analyses shown in this table indicate that the differences result from the butterflies being different (Column category) rather than the birds differing among themselves in their behavior towards the butterflies (Row category, $.25 > P > .10$). In order to make simultaneous comparisons between all the means by pairs, the *q* statistic was used with 95% confidence limits (Dixon & Massey, pp. 153-155). This analysis is shown in Table 5c where "yes" indicates that the paired means are significantly different, and "no" that they are not.

As shown above, *Parides* is completely unpalatable to the Blue Jays. *Danaus* and *Ithomia* are only slightly less so. *Heliconius sara* and *Lycorea* occupy an intermediate position between these species and the remaining five, *Biblis*, *Euptychia*, *Agraulis*, *Anartia* and *Victorina*. These latter five can be considered relatively palatable. The differences between the members of the unpalatable and palatable groups are all significant, but the differences within these two groups are not. The two intermediate species do not differ significantly from each other. However, *Lycorea* is more palatable than *Danaus*, although not significantly less acceptable than the five palatable species. This lack of significance results from the large variance in the treatment of *Lycorea*. *Heliconius sara* is significantly more palatable than *Danaus*, and significantly less palatable than four of the five palatable species, i.e., all except *Biblis*. Thus the breaks apparent in Table 4 are real, with the first three species not eaten 80% or more of the time, the last five eaten 80% or more of the time, while *Heliconius sara* and *Lycorea* were eaten about half the time. These results substantiate the classical statements in the literature to a remarkable extent.

2. The Species of Intermediate Palatability

Further examination of Table 4 indicates that the two species which are of intermediate palatability, *Lycorea* and *Heliconius sara*, have the greatest variances. This resulted from the large range in the frequencies not eaten by the five individual birds. These were .70 to .00 for *Lycorea* and .90 to .40 for *Heliconius sara*. While Bartlett's test has shown that the variances of the nine species as a group do not differ significantly, paired comparisons indicate that the variance of *Lycorea* is significantly higher than

TABLE 5. STATISTICAL ANALYSES OF THE DATA IN TABLE 4*

a. Homogeneity of the variances (Bartlett's test).

1. All the species:
F=3.23
d.f.=9/1375
P<.001
2. Nine species (excluding *Parides neophilus*):
F=1.01
d.f.=8/1163
.50>P>.25

b. Variance analysis of the nine means, excluding *Parides neophilus* (F test).

Variance categories	Sum of squares	Degrees of freedom	Mean square	F ratio	P
Birds (Rows)	1541	4	385	1.75	.25>P>.10
Butterflies (Columns)	28635	8	3579	16.27	<.001
Butterflies × Birds (Residual)	7046	32	220	—	—
TOTALS	37222	44	—	—	—

c. 95% confidence limits of the nine means by pairs, excluding *Parides neophilus* (q test).

	<i>Victorina</i>	<i>Anartia</i>	<i>Agraulis</i>	<i>Euptychia</i>	<i>Biblis</i>	<i>Lycorea</i>	<i>Heliconius</i>	<i>Ithomia</i>
<i>Danaus</i>	YES	YES	YES	YES	YES	YES	YES	NO
<i>Ithomia</i>	YES	YES	YES	YES	YES	NO	NO	
<i>Heliconius</i>	YES	YES	YES	YES	NO	NO		
<i>Lycorea</i>	NO	NO	NO	NO	NO			
<i>Biblis</i>	NO	NO	NO	NO				
<i>Euptychia</i>	NO	NO	NO					
<i>Agraulis</i>	NO	NO						
<i>Anartia</i>	NO							

"YES" indicates that the means are significantly different, "NO" that they are not

d. Variance analyses of the variances by pairs, excluding *Parides neophilus* (F test).

1. *Lycorea ceres* vs. *Biblis hyperia*:
F=12.43
d.f.=4/4
.05>P>.025
2. *Lycorea ceres* vs. *Danaus plexippus megalippe*:
F=10.13
d.f.=4/4
.05>P>.025
3. *Lycorea ceres* vs. *Victorina steneles*:
F=4.61
d.f.=4/4
.20>P>.10
4. All remaining paired comparisons:
Not significant by inspection.

*This analysis was performed on the data in Table 4 transformed according to the method of Snedecor, see text.

both that of *Danaus* in the unpalatable group and *Biblis* in the palatable group (Table 5d). Thus species that are either highly palatable or very unpalatable were treated uniformly. In contrast, the species of intermediate palatability were treated variably. The biological implications of this distinction are of great interest and will be discussed below (Section III-D-4).

(D). Discussion

1. Palatability Differences among the Species

The results of the experiment indicate that the ten species of butterflies fall into three groups

ranging from acceptable to completely unacceptable to the five Blue Jays (Table 4). The anticipated outcome was obtained for *Parides* and *Danaus*, close relatives of which had already been shown to be unpalatable to another species of jay (J. Brower, 1958a, b). *Ithomia* was also found to be unacceptable, confirming for one species the classical prediction that members of this subfamily would be distasteful. *Heliconius sara*, already demonstrated to be unpalatable to Silverbeak Tanagers (Brower, Brower & Collins, 1963), was also rejected more than half of the time by the Blue Jays. The finding that

Lycorea is intermediate was unexpected, because we anticipated that it would be closer to *Danaus* than it proved to be. The remaining five species were all relatively palatable to the Blue Jays.

2. Phylogenetic Correlations

For the theoretical reasons discussed by Brower, Brower & Collins (1963) (see also Section III-D-5), it was predicted that palatability, like morphology, would prove to be a more conservative taxonomic character in the Lepidoptera than color-pattern. On the basis of comparative morphology, it has been maintained that *Agraulis vanillae* is close to the nymphalid stock from which the Heliconiinae are derived. Because of this, the palatability of *A. vanillae* was predicted to be higher than that of the other heliconiines studied and in fact was shown to be (Brower, Brower & Collins, 1963). This has now been independently confirmed with Blue Jays which ate *A. vanillae* to a greater extent than *H. sara*. Similar confirmation of the accepted taxonomic scheme has been shown for *Lycorea* and *Danaus*, which belong to two tribes of the Danainae (Table 1). Following the classification of Forbes (1939), *Lycorea* is nearer to the ancestral nymphalid stock than *Danaus*, and the finding that *Lycorea* is the more palatable supports this. Another related pair, *Anartia* and *Victorina*, are in the tribe Vanessiini of the subfamily Nymphalinae. Although radically different in color-pattern, these two species are placed in adjacent genera by Seitz (1924) and were regarded as very closely related by W. Müller (1886). The fact that both are highly acceptable to the Blue Jays agrees with this. Finally, these two species, together with *Biblis*, support the hypothesis that the Nymphalinae in general are palatable.

Table 6 summarizes the comparative palatability of all the species which have been investigated to date in controlled, quantitative experiments. From this it can be seen that the present study, along with the previous ones, confirms the classical notions regarding the palatable and unpalatable groups of butterflies. Moreover, they establish that palatability is a valuable taxonomic character which ranks with morphology in determining phylogenetic relationships.

3. Potential Batesian Mimics

The results obtained also bear upon possible mimetic relationships between certain neotropical butterflies. On the basis of the finding that *Victorina s. steneles* is highly palatable, it can now be stated that it is a potential Batesian mimic of the heliconiine species *Philaethria dido* Clerck with which it is sympatric. *Philaethria* is bright green with black and brown mark-

ings. *Victorina* shares this color-pattern on the upper surface of its wings but, like many nymphalines, is cryptic on the undersurface. Thus if *Philaethria* proves to be distasteful, *Victorina* is a doubly protected species (Ford, 1955): by mimicry when flying, but by inconspicuousness when at rest.

In view of the fact that the nymphalines, *Biblis* and *Anartia*, are both palatable, their conspicuous coloration requires explanation because there are no known distasteful species which they resemble in detail. The alternative that their coloration functions solely as social releasers is unlikely since *Biblis* is a monotypic genus in Trinidad and apparently throughout its range, and *Anartia* includes only one other species in Trinidad besides *A. amalthea*, which is said to occupy a separate habitat (Kaye, 1921; Seitz, 1924). *Biblis* has a striking crimson border on the outer margin of black hindwings and solid black forewings. The upper surface of both sets of wings in *Anartia* bears a less discrete pattern of blackish-brown borders, white spots and single vertical red bands. The under surface of *Biblis* is similar to the upper surface, whereas that of *Anartia* is cryptic, being tan and mottled. The coloration of *Biblis*, and *Anartia* to a lesser extent, recalls in a general way that of the highly unpalatable Müllerian mimics, *Heliconius erato* hydara Hewitson and *Heliconius melpomene euryades* Riffarth. Both of these are velvet black with crimson patches on the forewings. There seems to be justification for the suggestion that *Biblis* and *Anartia* may derive some advantage from Batesian mimicry of these two *Heliconius*. This is based on the finding (Brower, Brower & Collins, 1963; J. Brower, 1963) that birds can generalize from experience with an unpalatable butterfly to reject on sight another which is broadly similar in size and shape, but totally unlike it in color-pattern. These two nymphalines could profitably be used in experiments to estimate the relative Batesian advantages of differing degrees of crude resemblance to a model such as *H. erato* or *H. melpomene*.

4. The Variable Treatment of Species of Intermediate Palatability and Its Bearing on the Dynamic Relationship of Batesian and Müllerian Mimicry

From an evolutionary point of view, a very interesting aspect of this study concerns the species of intermediate palatability which were treated in an extremely variable manner by the birds. A similar situation occurred in the earlier study (Brower, Brower & Collins, 1963), where the variances in treatment of the three least unpalatable heliconiines were seven or more times greater than those of the four very unpalatable

TABLE 6. SUMMARY OF COMPARATIVE PALATABILITY OF BUTTERFLIES IN CONTROLLED EXPERIMENTS WITH SINGLY-CAGED AVIAN PREDATORS

Species	Taxonomic Tribe	Predator	Per Cent Not Eaten				Reference
			Palatable	11-29	30-70	Unpalatable	
			<10	11-29	30-70	71-89	>90
<i>Papilio troilus</i> L.	Papilionini	Scrub Jay	X				J. Brower, 1958 b, c.
<i>Papilio palamedes</i> (Drury)	Papilionini	Scrub Jay	X				J. Brower, 1958 a, c.
<i>Papilio glaucus</i> L.	Papilionini	Scrub Jay	X				J. Brower, 1958 a, b, c.
<i>Papilio polyxenes</i> (Fabr.)	Papilionini	Scrub Jay	X				J. Brower, 1958 b.
<i>Battus philenor</i> L.	Troidini	Scrub Jay				X	J. Brower, 1958 b.
<i>Parides neophilus</i>	Troidini	Blue Jay				X	Present paper.
<i>Danaus p. plexippus</i> (L.)	Danaini	Scrub Jay				X	J. Brower, 1958 a.
<i>Danaus p. megalippe</i>	Danaini	Blue Jay				X	Present paper.
<i>Danaus gilippus berenice</i> (Cramer)	Danaini	Scrub Jay				X	J. Brower, 1958 c.
<i>Lycorea ceres</i>	Lycoreini	Blue Jay			X		Present paper.
<i>Ithomia drymo pellucida</i>	Ithomiini	Blue Jay				X	Present paper.
<i>Heliconius numata</i> Godart	Heliconiini	Silverbeak				X	Brower, Brower, & Collins, 1963.
<i>Heliconius melpomene</i>	Heliconiini	Silverbeak				X	Brower, Brower, & Collins, 1963.
<i>Heliconius erato</i>	Heliconiini	Silverbeak				X	Brower, Brower, & Collins, 1963.
<i>Heliconius sara</i>	Heliconiini	Silverbeak Blue Jay			X		Brower, Brower, & Collins, 1963. Present paper.
<i>Heliconius doris</i> (L.)	Heliconiini	Silverbeak				X	Brower, Brower, & Collins, 1963.
<i>Dryas iulia</i> (Fabr.)	Heliconiini	Silverbeak				X	Brower, Brower, & Collins, 1963.
<i>Agraulis vanillae</i> (L.)	Heliconiini	Silverbeak Blue Jay		X			Brower, Brower, & Collins, 1963. Present paper.
<i>Biblis hyperia</i>	Ergolini	Blue Jay		X			Present paper.
<i>Anartia amalthea</i>	Vanessini	Blue Jay	X				Present paper.
<i>Victorina steneles</i>	Vanessini	Blue Jay	X				Present paper.
<i>Limenitis a. archippus</i> (Cramer)	Limenitini (Nymphalinae)	Scrub Jay			X		J. Brower, 1958 a.
<i>Euptychia</i> spp.	Satyrini	Silverbeak Blue Jay	X		X		Brower, Brower, & Collins, 1963. Present paper.

species. This same tendency was also evident in another investigation where Scrub Jays freely ate *Papilio glaucus* Linnaeus, uniformly rejected *Danaus plexippus* (Linnaeus), and treated *Limnitis archippus archippus* (Cramer) variably, but as a group ate about half of them (J. Brower, 1958a). Since this appears to be a general phenomenon, it must be discussed further. Let us first investigate the possible reasons for the variable treatment and then consider its bearing on Batesian and Müllerian mimicry.

There are at least four explanations. First, individual butterflies could differ in their palatability through genetic polymorphism or continuous variation. There is no evidence for or against such genetically controlled differences but there are theoretical grounds for supposing that the scent of some species is polymorphic (Rothschild, 1961; Ford, 1964). F. Müller (1878) has noted a dimorphism of odor in the males of *Battus polydamus* (Linnaeus) and insofar as taste is partially dependent upon scent, dimorphism in flavor might also result. However, Müller's finding and palatability differences in general could also be explained (2) by environmentally determined differences among the individual butterflies, depending, for example, on the species of foodplant which their larvae had ingested in nature. There are several species of *Passiflora* available for the heliconiines in Trinidad (Alexander, 1961), and it is probable that *Lycorea* feeds upon more than one of the numerous Asclepiadaceae plants listed by Williams & Cheesman (1947). (We have observed it ovipositing and have reared several larvae to the adult stage on *Gonolobus broadwayi* Schltr., although several others given *Asclepias curassavica* Linnaeus under identical experimental conditions all died, L. Brower, unpub. data).

There is also the possibility that the birds differed in their taste sensitivity for either (3) genetic, or (4) environmental reasons. There is some critical evidence available that animals respond variably in their feeding preferences under experimental conditions. Kare & Ficken (1963) have shown that two-month-old domestic chickens exhibit marked differences in taste thresholds for various salt solutions, although importantly, irrespective of sex. They also discussed selective breeding experiments with chickens which indicated that the variation is under polygenic control. This seems true for saccharin preference in the rat as well (Nachman, 1959). Other experiments have shown variability, but have not determined whether it was genetically or environmentally controlled. Thus J. Brower (1960) found that some Starlings, *Sturnus vulgaris* Linnaeus, rejected food

treated with a particular concentration of quinine dihydrochloride, while others accepted it. Similarly, some toads, *Bufo terrestris* (Bonnaterre), freely ate stinging honeybees while others quickly learned to reject them (Brower & Brower, 1962). Other instances of differences in individual behavior towards identical food or flavored water solutions have been reported by Kare (1961) for the domestic chicken and game pheasant, by Kitchell (1961) for domestic pigeons, and by Duncan (1960a) for the feral pigeon. The latter author also found that certain pigeons are "taste-blind" to solutions which are normally repellent to other individuals (Duncan, 1960b; 1961). It is also clear that external and internal environmental differences can affect individuals' food preferences. Aside from the established fact that the rejection threshold for various noxious substances drops as the hunger of the animal increases, it is known that taste thresholds in both mammals and frogs vary with the temperature of the food as well as with atmospheric pressure (Moncrieff, 1951; Sato, 1963). Moreover, as Pfaffman (1963) has shown for rats, sensitivity towards food substances can be manipulated by what they are fed as well as by upsetting their hormone balance. Duncan (1960a, and b) also stated that hand-reared, naive, and wild-captured feral pigeons exhibit different taste preferences.

In the present study, it seems unlikely that the Blue Jays' taste thresholds varied for environmental reasons, because they were all maintained under comparable feeding and experimental conditions. Until further research is done, it does not seem possible to differentiate between the four alternatives. However, it is important to settle this matter because it may be the key to solving the problem of why certain presumably Müllerian species are polymorphic when theory predicts that they should be monomorphic so as to reduce the number of color-patterns that have to be remembered by the predators (Ford, 1964; Sheppard, 1963). Species of intermediate palatability may be so close to the threshold of being acceptable that under certain circumstances (e.g., the dry season, or other periods when alternative prey are less available) they may be taken, while at other times when food is more abundant they may be rejected. Consequently, they could alternate between being Batesian and Müllerian mimics, and if the former situation on the average prevailed, polymorphism would thereby be selectively advantageous.

5. Speculations on the Evolution of Unpalatability

Discussions of protective devices in animals

generally assume that they are products of specialization. Thus palatability is considered primitive and unpalatability specialized. It seems certain that this mode of defense has arisen independently several times in the butterflies, as is indeed suggested by perusal of Table 1. Moreover, it is probably still developing in certain lines, as Wallace (1889) suggested when discussing the Pieridae.

In this regard, the findings for *Lycorea* are of particular interest. This species is one of many belonging to the tiger-stripe complex in the neotropics which includes members of three groups, the Lycoreini, the Ithomiinae and the Heliconiinae (Kaye, 1906). It has always been maintained that these are all unpalatable and comprise a Müllerian relationship. This is supported by our findings, but we expected *Lycorea* to be as unpalatable as the others in these groups, which it is not. It is thus possible that the Lycoreini, the most primitive tribe of the Danainae (Forbes, 1939), are still in the process of evolving unpalatability and their mimicry of the other tiger groups is neither Batesian nor Müllerian but somewhere between these extremes.

On the other hand, it seems possible that *Lycorea* may represent an instance of the regression from unpalatability back towards palatability. The evolution of unpalatability must require complex readjustments in the physiology of a species so that the noxious chemical substances can be accommodated without poisoning the animal's own system, as discussed below (Section IV-D). Furthermore, even after the mechanisms have evolved, it is likely that they are to a certain extent detrimental to their possessors. Consequently, large selective forces favoring unpalatability must be required not only for its evolution, but also for its maintenance. Therefore if the advantage of unpalatability should lessen for any reason, it is possible that the genes controlling its development will be replaced by those which no longer produce it. This may have occurred for *Lycorea* as a consequence of the great number of unpalatable ithomiines and heliconiines which share its color-pattern. In this situation a species such as *Lycorea* could lose its unpalatability but retain the protective advantage of mimicry, particularly if it were rare. In other words, if the environment becomes saturated with many similarly colored, unpalatable species, the balance of advantage for unpalatability could be decreased to the extent that selection would favor a reversal from a Müllerian towards a Batesian status.

One way to test these alternatives is to study the species of the genus *Ituna* which are closely related to *Lycorea*, but are not involved in the

tiger mimicry complex, and the species of the genus *Clothilda* which are close to the nymphalid stem from which the other two genera have evolved. If *Lycorea* is more palatable than its close relatives but more unpalatable than the ancestral group, then regressive evolution is suggested. If they are about equally unpalatable and both less palatable than the ancestral group, then unpalatability is probably still in the process of evolution.

More generally, whereas regression of unpalatability may occur this way in certain of the exceptional Müllerian mimicry complexes, it probably occurs rarely in nature because of continual predation pressure. This is in fact supported by our findings that the unpalatability of species generally corroborates morphologically-based speculations on phylogeny. The theoretical interest of these possibilities associated with species of intermediate palatability emphasizes the dynamic relationship of Batesian and Müllerian mimicry and makes further research greatly to be desired.

IV. THE EVOLUTION OF PLANT POISONS AND THEIR BIOCHEMICAL FATE WHEN INGESTED

(A). *The General Problem*

Within ecological communities, there are three important ways in which the non-nutrient chemicals produced by plants may affect the animals. First, there is a large body of indirect evidence that many of these compounds have evolved as repellents to phytophagous insects and probably to other herbivorous invertebrates. Moreover, some of these repellents in the course of evolution appear to have been exploited secondarily as signal attractants and so operate in the physiological regulation of foodplant specificity (Dethier, 1954; L. Brower, 1958; Fraenkel, 1959; D. A. Jones, 1962).

Secondly, numerous plant components are known which are toxic or repulsive to herbivorous vertebrates. While there is a vast literature on the pharmacology, toxicology and medicinal effects of these chemicals on man and other mammals, there is little more than cursory discussion of the theory that (2) poisonous plant products have evolved as chemical repellents in response to the selection pressures exerted by all herbivorous vertebrates. Thus, the excellent discussion by Gundersen & Hastings (1944) on interdependence in plant and animal evolution did not develop the idea of this chemical relationship and this is true of the literature that we have examined in both general and specific fields

of plant and animal ecology, evolution, psychology, physiology and pharmacology³.

The third interaction involves three links in the foodchain—plants, herbivores and predators—rather than just plants and herbivores as in the first two interactions. The question is (3) have herbivorous vertebrates and invertebrates in the course of their evolution developed the ability to incorporate the poisonous and repellent plant chemicals, which have evolved as herbivore deterrents, and in turn use them to deter their own predators? The recent paper by Rothschild (1964) has come the closest to presenting this idea, but as far as we can determine, it has never been completely formulated in the literature; its proof will establish a new principle of biology. The remainder of this paper will attempt to elucidate various aspects of this third interaction, as exemplified by some phytophagous insects.

(B). *Evidence that the Larval Foodplants of Unpalatable Butterflies Contain Poisons*

Before we consider the mechanisms by which insects might utilize the secondary plant substances as part of their own defense against vertebrate predators, the literature will be reviewed to show that the larval foodplants of the five unpalatable groups of butterflies contain poisonous substances. These belong, as do many non-poisonous components, to a large class of compounds known as glycosides (Allport, 1943; McIlroy, 1951; Robinson, 1963). Glycosides consist of two major conjugates which, when hydrolyzed, break down to a sugar and an aglycone. In those that are poisonous, the aglycone may have quite different toxic effects from the glycoside itself. As will be seen below, the poisonous glycosides found in the foodplants of the unpalatable butterflies fall into four main categories: (1) *cyanogenic glycosides*, which on hydrolysis yield a cyanide-containing aglycone which in turn breaks down to form HCN; (2) *cardiac glycosides* (cardenolides), in which the aglycone (specifically called a genin) is a steroid that lacks nitrogen; (3) *saponins*, another special class of glycosides in which the aglycone is either a triterpenoid alcohol or a spiroketal steroid; (4) *alkaloids*, a heterogeneous group of usually basic compounds often occurring as glycosides in which the aglycone is a nitrogen-containing steroid.

Evidence that these poisonous substances occur in species from the various families to be discussed is based on a variety of source material

³Investigations of the physiological and psychological functions of the sense of taste in vertebrates could benefit greatly by being more cognizant of this ecological interaction with the plants (see Richter 1957; Teitelbaum & Epstein, 1963).

ranging from crude chemical tests in the field and information collected by naturalists on the uses of plants by local natives, to highly critical analyses carried out in modern laboratories. It is well to remember that the original discovery of medicinally valuable plants has depended principally upon their producing extreme physiological effects. Thus while repulsive, sickening or poisonous if eaten in quantity or without special preparation, when administered in sufficiently dilute concentration, they are either useful or at least produce pseudo-medicinal results. The poisonous properties of these plant substances are based largely upon their effects on mammals, and proof that they provide the chemical source of unpalatability in the insects awaits further investigation.

The Aristolochiaceae

According to Willaman & Schubert (1961), plants in this family contain at least ten different alkaloids, one of which, aristolochine, is said to cause cardiac and respiratory paralysis in mice as well as frogs (Henry, 1949). *Aristolochia rumicifolia* Mart. & Zucc. is used to make a curare arrow poison in South America (Henry, 1949). In India, members of the family are said to be nauseous and abortifacient (they are commonly called the birthworts), and are also used as insecticides (Chopra & Chopra, 1955). In Africa, the roots of one species, *Aristolochia albidia* Duchartre, are made into a weak, cold infusion and taken as a tonic, while the leaf is used for treatment of skin diseases and mixed with other components and drunk (Dalziel, 1937).

The Solanaceae

Even though plants of this family provide edible vegetables (potatoes, tomatoes and eggplant), they are generally narcotic and poisonous because of their contained alkaloids. These include the well-known and pharmacologically important parasymphathetic depressants from *Atropa belladonna* Linnaeus, as well as soladine and solanine from *Solanum*, and nicotine from *Nicotiana* (Allport, 1943; Chopra & Chopra, 1955; Willaman & Schubert, 1961). The poisonous properties of solanine were studied by Kline, von Elbe, Dahle & Kupchan (1961) who found that pregnant rats which had eaten sprouts of potato tubers gave birth to dead young or those which died in less than three days. The same effects were obtained when pure solanine was added to their diet, and it was concluded that the sprouts contained this alkaloid. Watt & Breyer-Brandwijk (1932) described the clinical effects of solanine which include vomiting, headache, diarrhea and colic, followed by depression. It is possible that the leaves of some species are edible, as Grisebach (1864) mentioned that *Solanum nodiflorum*

is eaten as a spinach, although Willaman & Schubert (1961) indicated that the fruits of this species contain solasodine. The fact that plants can be eaten as spinach may well result from the fact that the poisons in the leaves are extracted in the cooking water.

The Asclepiadaceae

These plants and their near relatives, the Apocyanaceae, are renowned for their numerous highly toxic cardiac glycosides (Abisch & Reichstein, 1962; Paris, 1963). Tschesche, Forstmann & Rao (1958) have isolated and partially identified seven different genins from *Asclepias curassavica*. This plant is native to tropical America, and in Trinidad we have reared several hundred larvae of *Danaus p. megalippe* and *D. gilippus xanthippus* Felder to the adult stage on it. According to Tschesche *et al.*, the usual extract from this plant is made from dried leaves and young parts of the stems, *i.e.*, precisely the parts which the larvae eat. Used in an infusion for pharmacological purposes, this acts as an excitant, an expectorant and a regulator of blood pressure, while the root extract is used as an emetic and a purgative. The *Asclepias* species in addition contain alkaloids as well as saponins (Henry, 1949; Chopra & Chopra, 1955). Whereas cardiac glycosides and their genins act, respectively, by affecting the heartbeat, and by causing convulsions, the general action of saponins is to irritate mucous membranes and to haemolyse red blood corpuscles when injected into higher animals. They act as highly toxic environmental poisons to fish (McIlroy, 1951). The aglycones of steroid saponins are also convulsants. Evidence of these toxic effects was given by Chopra & Chopra (1955) who stated that *A. curassavica* is used in India as a fish poison, an emetic and a cathartic. In Africa, Irvine (1961) noted that this species contains an active principle which causes death in large doses and is almost certainly poisonous to cattle.

Moreover, nicotine, a solanaceous alkaloid also found in *Asclepias* species, has an action on nerve ganglia resembling that of acetylcholine in producing a strong initial stimulation of the receptor, but differing in that the resultant depolarization is greatly protracted. Thus a temporary paralysis ensues (Cutting, 1962). Nicotine has therefore proved useful in the capture of wild animals. It is of interest in this context that in West Africa, Gilges (1955) reported that the natives in Balovale sprinkle the roots of *Asclepias lineolata* (Schltr.) among grain in order to catch birds, which become unconscious after eating them. On the other hand, Dalziel (1937) stated that the roots of the same species are used in an infusion to treat gastro-intestinal ailments.

This finding again emphasizes that although the plants are poisonous, they may be used medicinally if prepared and administered in sufficiently low concentrations. In addition, the young leaves, pods and shoots, as well as the tubers of some species of North American *Asclepias*, appear to be edible (Sturtevant, 1919; Watt & Breyer-Brandwijk, 1932). But as above, this is probably because the poisons are extracted in the cooking water. Another genus, *Calotropis*, is used to cause abortion, in homicide and suicide, and its "milk" is a drastic purgative (Chopra & Chopra, 1955). Tschesche, Snatzke & Grimmer (1959) have established that the genin from *Calotropis procera* known as calotropagenin is identical to asclepogenin from *A. curassavica*.

One of the best known members of the apocyanid group is the genus *Rauwolfia* which contains numerous poisonous alkaloids, including the tranquilizing drug, reserpine (Woodson, Youngken, Schlittler & Schneider, 1957; Irvine, 1961; Willaman & Schubert, 1961). Another apocyanid, *Nerium indicum* Mill., is used for suicide and abortion. In addition, *Nerium oleander* Linnaeus contains the glucosides neriin and oleandrin and is fatal to horses and cattle and to man even from meat skewered with the wood (Watt & Breyer-Brandwijk, 1932). Irvine (1961) has noted that many *Adenium* spp. are used in Africa to poison jackals and hyenas by baiting them to meat filled with a concoction of the plants. They are also used in arrow poison and to kill fish. These varied uses suggest the action of more than one type of poison, probably the cardiac glycosides in the baiting of mammals, and saponins for fish and poison arrows.

The Passifloraceae

These plants have been cited as non-poisonous by Poulton (1916), Sevastopulo (1952), and Brower, Brower & Collins (1963). Moreover, Henry (1949) did not include them in his book on alkaloids, nor did Muenscher (1939) in his book on poisonous plants. *Passiflora foetida* Linnaeus has edible fruit as do 16 other species of *Passiflora*, according to Sturtevant (1919). In fact, the leaves of *P. foetida* and its fruit when green contain cyanogenic glycosides as well as alkaloids and may be dangerous to stock (Dalziel, 1937; Arthur, 1954). Willaman & Schubert (1961) have in addition listed the alkaloid passiflorine from seven species, and Chopra & Chopra (1955) noted that this family produces hydrocyanic acid. Two species, *Adenia palmata* Engl. and *A. digitata* Burt Davy, have poisonous roots and fruits due to the presence of a cyanogenic glycoside and the toxalbumin, modeccin, (Watt & Breyer-Brandwijk, 1932). Another species, *Adenia lobata* (Jacq.) Engl., is said to be used

in arrow poison (Irvine, 1961). Thus the statements that the Passifloraceae are harmless were incorrect and the fact that the fruits are in some cases edible recalls the potato tuber which, too, is an unpoisonous part of an otherwise deadly plant.

From this survey, it can now be considered established that many poisonous chemicals occur in the plant families fed upon by the five major taxa of unpalatable butterflies. However, just as parts of these plants are sometimes edible, it is well to remember that most other plant families contain species, parts of which at some stage in their development contain poisonous substances (Willaman & Schubert, 1961). This includes some of the families fed upon by the palatable species investigated in this paper. The cited foodplant of *Biblis*, *Tragia volubilis* Linnaeus, is in the Euphorbiaceae and was considered by Grisebach (1864) to have an acrid and poisonous milky juice. The leaves of other species apparently produce hydrogen cyanide and according to Arthur (1954) may contain alkaloids. However, Webb (1955) studied 22 species from New Guinea in which no alkaloids were present and stated that plants of this family are certainly of negligible alkaloid interest. Many genera have multiple medicinal uses, and only the latex is stressed as a dangerous poison, while fruits and leaves are often eaten by antelopes and other animals (Irvine, 1961). Similarly palatable is the widely eaten rhizome, cassava, which comes from another member of this family, *Manihot esculenta* Crantz., although the fresh tubers are said to be extremely poisonous, and cassava has to be specially prepared before it is safe to eat (Chopra & Chopra, 1955).

Some of the plants belonging to the Acanthaceae, which contains the species eaten by *Victorina* and *Anartia*, are said to produce bitter or aromatic drugs (Grisebach, 1864) and others contain alkaloids (Arthur, 1954), while still others are also used medicinally (Irvine, 1961). On the other hand, the leaves of three genera are eaten as spinach and are not considered poisonous (Irvine, 1961). Furthermore, the Graminae, the foodplants of *Euptychia* spp., are generally edible to herbivores, but Chopra & Chopra (1955) have noted that some species of grass develop large quantities of hydrocyanic acid during dry periods.

It thus appears from this survey that the plant families of the palatable butterflies are not distinguished for poisonous properties, although exceptions exist, whereas among the foodplants of unpalatable butterflies, the reverse is true, most being poisonous. Clearly, more critical evidence is needed, and while these generalizations

about plant families are valuable, quantitative and qualitative analyses of the particular species of plant fed upon by the insects must be made. Furthermore, the actual parts of the plants eaten must be analyzed, and these should be taken from the plants at a time which corresponds to the natural feeding period of the insects. For as Flück (1963) has pointed out, secondary substances may be synthesized in one part of a plant but concentrated in another, and variation in the amounts of the chemicals in any one tissue occurs seasonally, daily, and according to the physiological state of the plant.

(C). Utilization of the Plant Poisons

There are several ways in which ingested poisonous plant substances might be utilized as predator deterrents. Let us consider these for the phytophagous insects in order of increasing complexity. Defense could result simply by (1) *the accumulation of the plant chemicals in the gut*. This idea was first tested by Eltringham (1909, 1910) who found that larvae of the moth *Odontopera* (= *Gonodontis*) *bidentata* Clerck were extremely distasteful to lizards, *Lacerta viridis*, when fed ivy, but when subsequently given apple for a period of time, became palatable. Recently Eisner, Kafatos & Linsley (1962) have extended this to a fourth link in the foodchain by suggesting that cerambycid beetles of the genus *Elytroleptus* may become temporarily unpalatable by eating lycid beetles, *Lycus* spp., which themselves are distasteful. This mechanism could suffice for insects which continue throughout their lives to eat poisonous foods, but it would not account for the unpalatability of those such as adult Lepidoptera, which undergo a radical change in food habits after metamorphosis, either shifting to nectar or ceasing to eat altogether.

Consequently, an alternative mechanism is required which could be (2) *the assimilation and storage of poisonous molecules generally throughout the blood or other body tissues*. Meldola (1877) first suggested this and it is still held possible by modern workers (Eisner, Kafatos & Linsley, 1962). The first support came from the work of F. M. Jones (1937). Using three species of *Asclepias*-eating beetles, *Tetrapopes tetraophthalmus* Frst. (Cerambycidae), and *Chrysochus auratus* Fabr., and *Labidomera clivicollis* Kby. (Chrysomelidae), he found that individuals which had recently been fed the plant and those which had been starved for five days to clear the gut were rejected by both birds and ants, even though these predators ate control food items. A more direct line of evidence was produced by D. A. Jones, Parsons & Rothschild (1962) who showed that the tissues

in all stages of two species of zygaenid moths produce hydrocyanic acid when crushed, and a precursor of this poison, a cyanogenic glucoside, is also present in their foodplants. However, this example is complicated by the fact that the same insects produce HCN when fed upon an acyanogenic strain of the same plant, as will be discussed in Section IV-E.

The most specialized mechanism could be (3) *the assimilation, concentration and storage of the poisonous molecules in glands*. Roth & Eisner (1962) agree with the probable existence of this mechanism, but there are in fact no certain demonstrations of it. The most likely example comes from the work of Waterhouse, Forss & Hackman (1961) who have shown that a coreid stink-bug, *Amorbus rubiginosus* Guer., produces 2-hexenal, an aldehyde identical or chemically similar to that found in several plant species including *Eucalyptus* upon which they feed. It is almost certainly defensive since it is effective in repelling ants in the laboratory, and related but unidentified pentatomid and coreid bugs deter ants, amphibia, birds and mice. The cockroach, *Eurycotis floridana* (Walker), also produces 2-hexenal (Roth, Niegisch & Stahl, 1956), as do two other phytophagous bugs, *Brochymena quadripustulata* (Fabr.) and *Acanthocephala femorata* (Fabr.) (Blum, Crain & Chidester, 1961; Blum, 1961). However, once again the evidence that they concentrate the aldehyde from their food is only correlative. Another possible case was discussed by Pavan (1959). He proposed a biochemical scheme by which salicyl aldehyde from the dorsal gland of the beetle larva, *Melasma populi* Linnaeus, might be constructed from components present in its foodplant, willow (Salicaceae).

Less direct evidence that the insects utilize the plant substances also exists. Two highly pertinent studies on *Asclepias*-feeding grasshoppers have been made. Steyn (1962) reported detailed observations on the death of a four-year-old Bantu child within 12 hours after she had eaten a single adult *Phymateus leprosus* Fabr. Symptoms included extreme vomiting and salivation, followed by respiratory and cardiac failure. In subsequent experiments with adult rabbits, he found that 90 grams of freshly minced grasshoppers suspended in water and fed by stomach tube also caused respiratory and cardiac deterioration and death. In nature this species is known to feed on *Asclepias fruticosa* and *Nerium oleander*. More recently Parsons (1963, and personal communication) has reported that the secretion of one individual of *Poekilocerus bufonius* Klug is sufficient to kill several cats when injected intravenously. The poison, which he is

investigating (Rothschild & Parsons, 1962), appears to be digitalis-like in its action and may be a cardiac glycoside similar to that found in its foodplant, *Asclepias curassavica*.

An additional line of indirect evidence has been cited by McCann (1953) who described an Indian grasshopper, *Aularches miliaris* (Linnaeus), which as nymphs eat the pungent *Heterophragma roxburghii* A. de C. (Bignoniaceae) and emit an odor similar to that of the leaves. As adults, they change their feeding habits and eat the highly poisonous and acrid leaves of *Gloriosa superba* Linnaeus (Liliaceae), and now emit a fluid with a corresponding odor.

Finally, the correlation between the unpalatable butterflies and their poisonous foodplants has two remarkable parallels in the Hemiptera of the New World. The lygaeid bugs, *Onchopeltus fasciatus* (Dallas) and *Lygaeus kalmii* Stal., feed upon *Asclepias* and are highly distasteful to lizards, Scrub Jays and ants (F. M. Jones, 1932; Parsons, 1940; J. Brower, unpub. data). In Trinidad two warningly colored and gregarious coreids, *Leptoglossus (Diactor) bilineatus* Fabr. and *Anisocelis (Hymeniphora) hymeniphora* Westwood, live upon *Passiflora serrato-digitata* Linnaeus which they almost certainly eat (Brower & Brower, personal observations, summer, 1963).

(D). Mechanisms for Preventing Autotoxicity

In order for herbivores to utilize the plant poisons in their own defense, they must have mechanisms to prevent themselves from being poisoned, as is indeed implied by their very ability to feed upon the plants containing these substances. A large body of biochemical evidence has shown that both vertebrates and invertebrates are able to transform poisonous molecules into non-poisonous ones either when taken orally or injected. Detoxification more often than not results in increasing the size of the molecule rather than in breaking it down, and the products can be accumulated in a harmless form in the tissues of the animals, as well as excreted or defecated (Smith, 1955; Brodie, 1956; Gilmour, 1961).

If the defense were simply by accumulation in the gut (method 1 above), no metabolic reworking of the poisons would be required, provided that the insect itself were resistant to the chemical. In this instance the plant poison would be transferred directly to the predator. On the other hand, modern findings in the field of detoxification biochemistry as discussed by Williams (1959) make it more likely that the substance would be built into a harmless compound in the gut, but would break down again when ingested by a predator. Similarly detoxified

compounds could be distributed throughout the body tissue or hemolymph (method 2), or concentrated in glands (method 3) and again break down to yield the active poisons when eaten by the predator.

The recent study of Remold (1963) is of interest in showing how autotoxicity is avoided by mechanical means in the pentatomid bug *Carpocoris purpureipennis*, which secretes a defensive aldehyde in specialized glands. When sprayed by the bug on ants or other insects, this substance immediately flows over their cuticular surface and acts as a contact poison, causing paralysis. Moreover, it also repels them from a distance. Puncturing the reservoir of an individual bug resulted in releasing the secretion into its hemolymph, which caused immediate paralysis, as did allowing it to flow over the surface of the bug's body after abrading the cement layer. Thus these bugs' own chemical defense system is highly poisonous, but is kept harmless by storage in the reservoir gland, and by impermeability of their intact cuticle. Moreover, entrance of the substance into their own tracheae is prevented by specialized mushroom-shaped cuticular structures near their spiracles, which greatly increase the evaporative surface.

Another instance where a defensive secretion of an insect has been proven autotoxic is in the case of the cockroach, *Eurycotis floridana*, mentioned above (Roth, Niegisch & Stahl, 1956). Similarly, Waterhouse, Forss & Hackman (1961) showed that the coreid stink-bug, *Rhoeocoris*, dies if the insects are crowded together without ventilation. In addition, some insects synthesize toxic substances *de novo* which they store in a harmless form through conjugation with glucose to form beta-glucosides (Brunet, 1963a, b; Brunet & Kent, 1955). Other examples of autotoxic secretions are given by Pavan (1959), Crescitelli & Geissman (1962) and Roth & Eisner (1962).

It is important to realize that some species of phytophagous insects which feed upon poisonous plants are cryptic and for this reason have been assumed to be palatable (Sevastopulo, 1952). It is possible that some of these are adapted to destroy, excrete, defecate or convert the poisons into permanently detoxified compounds rather than secondarily utilizing them to build up their own chemical defense systems. Others may still be in the process of evolving unpalatability as discussed in Section III-D-5, and may not yet have evolved a warning coloration. However, some species, such as the larvae of the moth *Mania* (= *Phalaena*) *typica* Linnaeus, are both extremely unpalatable and highly cryptic (Poulton, 1887; Eltringham, 1909).

Consequently, cryptic coloration should not always be interpreted to indicate palatability any more than conspicuous coloration always implies the reverse, as for example, in Batesian mimics, or in species such as the moth, *Rhododipsa masoni* J. B. Smith, which, though yellow and scarlet, inhabits similarly colored flowers and is therefore in fact cryptic (Brower & Brower, 1956).

Finally it is of great interest that insects differ basically from vertebrates, but are similar to plants, in the way in which they detoxify certain natural products by conjugation with sugar or sugar derivatives: vertebrates form glucuronic acids or glucuronides, while insects and plants form glycosides (Williams, 1959; Pridham, 1960). This suggests that the biochemical machinery of the insects is closely adapted to that of plants and in our opinion increases the likelihood that the insects can utilize the poisons from the plants rather than having to produce them by *de novo* synthesis.

(E) The Alternative of *de novo* Synthesis of the Defensive Poisons

Roth & Eisner (1962) have suggested that the defensive secretions of most arthropods are probably produced metabolically, that is to say, through *de novo* synthesis. For example, the quinones are widely used in defense, and as Brunet (1963a, b) has shown, these most often seem to be synthesized from the amino acids tyrosine and phenylalanine. The production of hydrocyanic acid in zygaenid moths may also be metabolic, instead of derived from the plant as discussed above. Even when fed upon strains of plants in which the cyanogenic glycoside has not been detected, the moths still produced it when their tissues were crushed (D. A. Jones, *et al.*, 1962). It seems to us that more work is needed on this problem, for the reason that the glycoside may occur in the plant in such a low concentration as to be difficult to detect, and yet be concentrated by the insects. A similar criticism can be directed towards another elegant investigation. In attempting to shed light on the biochemical origin of aldehydes in the Hemiptera, Gordon, Waterhouse & Gilby (1963) injected green vegetable bugs, *Nezara viridula* (Fabr.) (Pentatomidae), with radioactive sodium acetate. The purpose of the experiment was to find out whether or not the species could utilize the acetate molecule as a building unit in the synthesis of the aldehydes and thus prove that the repellent substance need not be concentrated from the plants. The results indicated beyond doubt that the radioactive carbon ended up in the bugs' repellent secretion. However, the experiment by-passed the digestive

system and does not disprove that the bugs may be able to incorporate the aldehydes from the plants they eat.

Although the idea of *de novo* synthesis, originally put forward by Meldola (1877), is of undoubted importance, we suspect that further research will establish that the poisonous molecules in the plants are more often utilized by the insects in biochemical defense than is at present thought to be the case. This prediction is made more plausible when one considers that carotenoids are synthesised only by plants, yet are often in the integumental coloring of insects (Cromartie, 1959; Fox & Vevers, 1960) and form the biochemical basis of the visual systems of both vertebrates and invertebrates (Gilmour, 1961; Prosser & Brown, 1961).

V. CONCLUSION

While correlation does not prove causation, we believe that the facts, as presented in this paper, make a casual relation almost certain, *i.e.*, the adult butterflies of five major taxa are unpalatable to vertebrate predators because their larvae are able to incorporate into their own tissues the unpalatable and poisonous molecules produced by the plants.

One approach to obtaining proof of this relationship would be to offer to vertebrate predators the adults of a polyphagous butterfly species whose larvae had been fed either poisonous or non-poisonous foodplants. Analyses could then be undertaken if a significant difference were found between the two groups. A species which appears to be particularly suitable is *Euptoieta claudia* Cramer, a member of the tribe Argynnini near the nymphalid stem, which on the one hand has given rise to the palatable nymphalines and on the other to the unpalatable heliconiines. This butterfly is reported to feed on plants from a number of families, including the Violaceae which are the main food of the palatable argynnids, and the Passifloraceae, the foodplants of the heliconiines and acraeids. Indeed, Hasse (1896) suggested that the most primitive heliconiine genera have become unpalatable by changing their food from related families such as Violaceae to the Passifloraceae. A second approach would be to induce a known unpalatable species to eat a non-poisonous foodplant and determine if it then becomes palatable. For example, the milkweed bug, *Onchopeltus fasciatus*, can be fed a variety of edible seeds including raw peanuts (Frings & Peissner, 1952). Alternatively, synthetic diets could be used (see House, 1961).

With the analytical tools now available in biology, including chromatography and labelling

with radioactive substances, the study of the transfer of chemical molecules from the plant through the herbivore has become an exciting prospect. It will be a fitting reward to the efforts of naturalists over the past hundred years if these new methods prove their speculations correct.

VI. SUMMARY

1. Representative species from four of the five major groups of butterflies (Troidini, Heliconiinae, Danainae and Ithomiinae) classically held to be unpalatable to vertebrate predators were quantitatively demonstrated to be unacceptable to five experimental Blue Jays, *Cyanocitta cristata bromia*, and those from two other groups (Satyrinae and Nymphalinae) held to be palatable proved to be so.

2. The foodplants of the distasteful groups of butterflies belong, respectively, to the families Aristolochiaceae, Passifloraceae, Asclepiadaceae and Solanaceae. These are all characterized by their contained poisonous substances and contrast with the foodplants of the edible groups of butterflies which in general are not poisonous.

3. A large body of correlative evidence is presented in support of the theory that plants which contain substances repellent or toxic to herbivorous vertebrates and invertebrates impart to specific phytophagous insects their poisonous molecules which these insects in turn utilize in their own defense against vertebrate predators.

4. It is thus thought that the specific chemical substances which cause adult butterflies to be unpalatable are derived from the poisons in the larval foodplants rather than synthesized *de novo* by the insects.

5. The experiment established that comparative palatability of species ranks with morphology in assessing phylogenetic relationships.

6. The Blue Jays treated both the very palatable and the very unpalatable species uniformly, whereas individual birds tended either to eat or reject those of intermediate palatability. This variable treatment of the intermediate species is interpreted as a threshold phenomenon and may explain why certain Müllerian mimics are polymorphic.

7. The evolution and maintenance of unpalatability, which is of advantage in reducing predation, is considered to be physiologically detrimental. Consequently, in certain species of large Müllerian associations, the balance of advantage for unpalatability could be decreased to an extent that selection would favor a reversal from a Müllerian towards a Batesian status.

VII. REFERENCES

ABISCH, EVA, & T. REICHSTEIN

1962. Orientierende chemische Untersuchung einiger Asclepiadaceen und Periplocaceen. *Helvetica Chimica Acta*, 45: 2090-2116.

ALEXANDER, ANNE J.

1961. A study of the biology and behavior of the caterpillars, pupae and emerging butterflies of the subfamily Heliconiinae in Trinidad, West Indies. Part I. Some aspects of larval behavior. *Zoologica*, 46: 1-24.

ALLPORT, N. L.

1943. The chemistry and pharmacy of vegetable drugs. George Newnes Ltd., London. 252 pp.

ARTHUR, H. R.

1954. A phytochemical survey of some plants of North Borneo. *Jour. Pharmacy and Pharmacology*, 6: 66-72.

BATES, H. W.

1862. Contributions to an insect fauna of the Amazon Valley. *Lepidoptera: Heliconidae*. *Trans. Linnaean Soc. London*, 23: 495-566.

BEDDARD, F. E.

1892. *Animal Coloration*. Swan Sonnenschein & Co., London. 288 pp.

BISSET, G. W., J. F. D. FRAZER, MIRIAM ROTHSCHILD & M. SCHACHTER

1960. A pharmacologically active choline ester and other substances in the garden tiger moth *Arctia caja* (L.). *Proc. Roy. Soc., B*, 152: 255-262.

BLUM, M. S.

1961. The presence of 2-hexenal in the scent gland of the Pentatomid *Brochymena quadripustulata*. *Annals of the Entomological Soc. of America*, 54: 410-412.

BLUM, M. S., R. D. CRAIN & J. B. CHIDESTER

1961. Trans-2-hexenal in the scent gland of the Hemipteran *Acanthocephala femorata*. *Nature*, 189: 245-246.

BRODIE, B. B.

1956. Pathways of drug metabolism. *Jour. Pharmacy and Pharmacology*, 8: 1-17.

BROWER, JANE V. Z.

- 1958a. Experimental studies of mimicry in some North American butterflies. Part I. The Monarch, *Danaus plexippus*, and Viceroy, *Limenitis archippus archippus*. *Evolution*, 12: 32-47.
- 1958b. Experimental studies of mimicry in some North American butterflies. Part II. *Battus philenor* and *Papilio troilus*, *P. polyxenes*, and *P. glaucus*. *Evolution*, 12: 123-136.

1960. Experimental studies of mimicry. Part IV. The reactions of Starlings to different proportions of models and mimics. *Amer. Naturalist*, 94: 271-282.

1963. Experimental studies and new evidence on the evolution of mimicry in butterflies. Symposium on Mimicry, Proc. XVI International Congress of Zoology, Washington, D. C., Vol. 4: 156-161.

BROWER, L. P.

1958. Bird predation and foodplant specificity in closely related procrystic insects. *Amer. Naturalist*, 92: 183-187.

1963. The evolution of sex-limited mimicry in butterflies. Symposium on Mimicry, Proc. XVI International Congress of Zoology, Washington, D. C., Vol. 4: 173-179.

BROWER, JANE V. Z., & L. P. BROWER

1962. Experimental studies of mimicry. 6. The reaction of toads (*Bufo terrestris*) to honeybees (*Apis mellifera*) and their dronefly mimics (*Eristalis vinetorum*). *Amer. Naturalist*, 96: 297-307.

BROWER, L. P., & FLORENCE P. CRANSTON

- (In press). A quantitative study of courtship behavior in the Queen butterfly, *Danaus gilippus berenice*.

BROWER, L. P., & JANE V. Z. BROWER

1956. Cryptic coloration in the anthophilous moth *Rhododipsa masoni*. *Amer. Naturalist*, 90: 177-182.

BROWER, L. P., JANE V. Z. BROWER & C. T. COLLINS

1963. Experimental studies of mimicry. 7. Relative palatability and Müllerian mimicry among neotropical butterflies of the subfamily Heliconiinae. *Zoologica*, 48: 65-84.

BRUNET, P. C. J.

- 1963a. Tyrosine metabolism in insects. *Ann. New York Acad. Sciences*, 100: 1020-1034.

- 1963b. Synthesis of an aromatic ring in insects. *Nature*, 199: 492-493.

BRUNET, P. C. J., & P. W. KENT

1955. Mechanism of sclerotin formation: the participation of a beta-glucoside. *Nature*, 175: 819.

BUTLER, A. G.

1869. Remarks upon certain caterpillars etc. which are unpalatable to their enemies. *Trans. Ent. Soc. London*, 1869: 27-29.

CARPENTER, G. D. H.

1921. Experiments on the relative edibility of insects, with special reference to their coloration. *Trans. Ent. Soc. London*, 1921: 1-105.

1942. Observations and experiments in Africa by the late C. F. M. Swynnerton on wild birds eating butterflies and the preference shown. *Proc. Linn. Soc. London*, 154: 10-46.

CHOPRA, R. N., & I. C. CHOPRA

1955. A review of work on Indian medicinal plants. Indian Council of Medical Research. Special Report No. 30, New Delhi. 263 pp.

CLARK, A. H., & LEILA F. CLARK

1951. The butterflies of Virginia. Publ. by Smithsonian Institution. Publ. 4050. Smithsonian Misc. Coll., Vol. 116: 1-239, 30 plates.

CRANE, JOCELYN

1954. Spectral reflectance characteristics of butterflies (Lepidoptera) from Trinidad, B. W. I. *Zoologica*, 39: 85-115, 2 pls.

CRESCITELLI, F., & T. A. GEISSMAN

1962. Invertebrate pharmacology: selected topics. *Ann. Review of Pharmacology*, 2: 143-192.

CROMARTIE, R. I. T.

1959. Insect pigments. *Annual Review of Entomology*, 4: 59-76.

CUTTING, W. C.

1962. Handbook of pharmacology, the actions and uses of drugs. Appleton-Century-Crofts, New York. 643 pp.

DALZIEL, J. M.

1937. The useful plants of West Tropical Africa. The Crown Agents for the Colonies, London. 612 pp.

DARWIN, C.

1871. The descent of man and selection in relation to sex. Murray, London. Vol. 1, pp. 415-417.

DETHIER, V. G.

1954. Evolution of feeding preferences in phytophagous insects. *Evolution*, 8: 33-54.

DIXEY, F. A.

1894. On the phylogeny of the Pierinae. *Trans. Ent. Soc. London*, 1894: 249-334.

DIXON, W. J., & F. J. MASSEY, JR.

1957. Introduction to statistical analysis. McGraw-Hill Book Co., Inc., New York. 488 pp.

DUNCAN, C. J.

- 1960a. Preference tests and the sense of taste in the feral pigeon (*Columba livia* Var Gmelin). *Animal Behavior*, 8: 54-60.
1960b. The sense of taste in birds. *Ann. Applied Biology*, 48: 409-414.
1961. Taste in relation to food preference. *The Poultry Review*, 1: 55-59.

EHRlich, P. R.

1958. The comparative morphology, phylogeny and higher classification of the butterflies (Lepidoptera: Papilionoidea). *Univ. Kansas Science Bull.*, 39: 305-370.

EISNER, T., F. C. KAFATOS & E. G. LINSLEY

1962. Lycid predation by mimetic adult Cerambycidae (Coleoptera). *Evolution*, 16: 316-324.

ELTRINGHAM, H.

1909. An account of some experiments on the edibility of certain Lepidopterous larvae. *Trans. Ent. Soc. London*, 1909: 471-478.
1910. Edibility of Lepidopterous larvae. *Proc. Ent. Soc. London*, 1910: xxxi-xxxii.

FAWCETT, J. M.

1901. Notes on the transformation of some South-African Lepidoptera. *Trans. Zool. Soc. London*, 15: 291-322.
1903. Notes on the transformation of some South-African Lepidoptera. *Trans. Zool. Soc. London*, 17: 165-190.

FINN, F.

1895. Contributions to the theory of warning colours and mimicry. I. Experiments with a Babbler (*Crateropus canorus*). *Jour. Asiatic Soc. Bengal*, 64: 344-356.
1896. Contributions to the theory of warning colours and mimicry. II. Experiments with a lizard (*Calotes versicolor*). *Jour. Asiatic Soc. Bengal*, 65: 42-48.
1897a. Contributions to the theory of warning colours and mimicry. III. Experiments with a tupaia and a frog. *Jour. Asiatic Soc. Bengal*, 66: 528-533.
1897b. Contributions to the theory of warning colours and mimicry. IV. Experiments with various birds. *Jour. Asiatic Soc. Bengal*, 66: 613-668.

FISHER, R. A.

1958. The genetical theory of natural selection. 2nd revised ed. Dover Publ., Inc., New York. 291 pp.

FLÜCK, H.

1963. Intrinsic and extrinsic factors affecting the production of secondary plant products. In *Chemical plant taxonomy*, ed. T. Swain, Academic Press, London. pp. 167-186.

FORBES, W. T. M.

1939. Revisional notes on the Danainae (Lepidoptera). *Entomologica Americana*, 19 (new series): 101-140.

FORD, E. B.

1955. Moths. Collins, London. 266 pp.
1964. *Ecological Genetics*. Methuen & Co. Ltd., London. 335 pp.

FOWLER, K. S., & S. E. LEWIS

1958. The extraction of acetylcholine from frozen insect tissue. *Jour. of Physiology*, 142: 165-172.

FOX, H. MUNRO & GWYNNE VEVERS

1960. The nature of animal colours. Sidgwick & Jackson, Ltd., London. x + 246 pp.

- FRAENKEL, G. S.
1959. The raison d'être of secondary plant substances. *Science*, 129: 1466-1470.
- FRAZER, J. F. D., & MIRIAM ROTHSCHILD
1960. Defence mechanisms in warningly-coloured moths and other insects. XI International Congress for Entomology, Vienna. Symposium 4, Vol. 3: 249-256.
- FRINGS, H., & LORRAINE PEISSNER
1952. Substitutes for milkweed seeds in the rearing of milkweed bugs. *Jour. Economic Entomology*, 45: 752.
- GILGES, W.
1955. Some African poison plants and medicines of Northern Rhodesia. No. 11. Occasional papers of the Rhodes-Livingstone Museum, Livingstone, Northern Rhodesia. 33 pp.
- GILMOUR, D.
1961. The biochemistry of insects. Academic Press, New York. xii + 343 pp.
- GOOD, R.
1947. The geography of the flowering plants. Longmans, Green & Co., London. 403 pp.
- GORDON, H. T., D. F. WATERHOUSE & A. R. GILBY
1963. Incorporation of ^{14}C -acetate into scent constituents by the green vegetable bug. *Nature*, 197: 818.
- GRISEBACH, A. H. R.
1864. Flora of the British West Indian islands. Lovell Reeve & Co., London. 789 pp.
- GUNDERSEN, A., & G. T. HASTINGS
1944. Interdependence in plant and animal evolution. *The Scientific Monthly* (A. A. A. S.), July: 63-72.
- HAASE, E.
1896. Researches on mimicry on the basis of a natural classification of the Papilionidae. Pt. II. Transl. by C. M. Childs. Nagele, Stuttgart. 154 pp., 8 col. pls.
- HENRY, T. A.
1949. The plant alkaloids. J. and A. Churchill Ltd., London. 804 pp.
- HOUSE, H. L.
1961. Insect nutrition. *Annual Review of Entomology*, 6: 13-26.
- HUHEEY, J. E.
1961. Studies in warning coloration and mimicry. III. Evolution of Müllerian mimicry. *Evolution*, 15: 567-568.
- IRVINE, F. R.
1961. Woody plants of Ghana. Oxford University Press, London. 868 pp.
- JONES, D. A.
1962. Selective eating of the acyanogenic form of the plant *Lotus corniculatus* L. by various animals. *Nature*, 193: 1109-1110.
- JONES, D. A., J. PARSONS & MIRIAM ROTHSCHILD
1962. Release of hydrocyanic acid from crushed tissues of all stages in the life-cycle of species of the Zygaeninae (Lepidoptera). *Nature*, 193: 52-53.
- JONES, F. M.
1932. Insect coloration and the relative acceptability of insects to birds. *Trans. Ent. Soc. London*, 80: 345-385.
1937. Relative acceptability and poisonous food-plants. *Proc. Roy. Ent. Soc. London*, 12: 74-76.
- KARE, M. R.
1961. Comparative aspects of the sense of taste. In M. R. Kare and B. P. Halpern, *Physiological and behavioral aspects of taste*. Univ. Chicago Press, Chicago. 149 pp.
- KARE, M. R., & M. S. FICKEN
1963. Comparative studies on the sense of taste. In *Olfaction and taste*, ed. Y. Zotterman. Pergamon Press, London. pp. 285-297.
- KAYE, W. J.
1906. Notes on the dominant Müllerian group of butterflies from the Potaro district of British Guiana. *Trans. Ent. Soc. London*, 1906: 411-439.
1921. A catalogue of the Trinidad Lepidoptera. Rhopalocera (butterflies). *Memoirs Dept. Agriculture Trinidad & Tobago*. No. 2, 163 pp.
- KITCHELL, R. L.
1961. Neural response patterns in taste. In M. R. Kare and B. P. Halpern, *Physiological and behavioral aspects of taste*. Univ. Chicago Press, Chicago. pp. 39-49.
- KLINE, B. E., H. VON ELBE, N. A. DAHLE & S. M. KUPCHAN
1961. Toxic effects of potato sprouts and of solanine fed to pregnant rats. *Proc. Soc. Exptl. Biol. and Med.*, 107: 807-809.
- KLOTS, A. B.
1933. A generic revision of the Pieridae (Lepidoptera). *Entomologica Americana*, 12 (new series): 139-242.
- MARSHALL, G. A. K.
1902. Five years' observations and experiments (1896-1901) on the bionomics of South African insects, chiefly . . . mimicry and warning colours. *Trans. Ent. Soc. London*, 1902: 287-584.
- MCCANN, C.
1953. Aposematic insects and their food plants. *Jour. Bombay Nat. Hist. Soc.*, 51: 752-754.

- McILROY, R. J.
1951. The plant glycosides. Edward Arnold and Co., London. 138 pp.
- MELDOLA, R.
1877. No title. Proc. Ent. Soc. London, 1877: xii.
- MONCRIEFF, R. W.
1951. The chemical senses. Leonard Hill Ltd., London. 538 pp.
- MUENSCHER, W. C.
1939. Poisonous plants of the United States. Macmillan Co., New York. xvii + 266 pp.
- MÜLLER, F.
1878. Notes on Brazilian entomology. Trans. Ent. Soc. London, 1878: 211-223.
- MÜLLER, W.
1886. Südamerikanische Nymphalidenraupen. Jena. Gustav Fischer. x + 255 pp. 4 pls.
- MUNROE, E.
1960. The classification of the Papilionidae (Lepidoptera). The Canadian Entomologist, Supplement 17 of Vol. 92: 3-51.
- MUNROE, E., & P. R. EHRLICH
1960. Harmonization of concepts of higher classification of the Papilionidae. Jour. Lepidopterists' Soc., 14: 169-175.
- NACHMAN, M.
1959. The inheritance of saccharin preference. Jour. of Comp. and Physiological Psychology, 52: 451-457.
- PARIS, R.
1963. The distribution of plant glycosides. In Chemical plant taxonomy, ed. T. Swain, Academic Press, London. pp. 337-358.
- PARSONS, C. T.
1940. Observations in Cuba on insect mimicry and warning coloration. Psyche, 47: 1-7.
- PARSONS, J.
1963. Heart poison from a toxic grasshopper. Biochemical Pharmacology 12. Supplement: 126-127.
- PAVAN, M.
1959. Biochemical aspects of insect poisons. IVth International Congress of Biochemistry, Vienna, 1958, 12: 15-36.
- PFAFFMANN, C.
1963. Taste stimulation and preference behavior. In Olfaction and taste, ed. Y. Zotterman. Pergamon Press, London. pp. 257-273.
- POCOCK, R. I.
1911. On the palatability of some British insects, with notes on the significance of mimetic resemblances. Notes by E. B. Poulton. Proc. Zoological Soc. London, 1911: 809-868.
- POULTON, E. B.
1887. The experimental proof of the protective value of colour and markings in insects in reference to their vertebrate enemies. Proc. Zool. Soc. London, 1887: 191-274.
1916. Haase's hypothesis that distasteful qualities of Lepidoptera are derived direct from the food plant. Proc. Ent. Soc. London, 1916: lxx-lxxi.
- PRIDHAM, J. B.
1960. The formation and possible function of phenolic glycosides. In Phenolics in plants in health and disease. ed. J. B. Pridham. Pergamon Press, London. pp. 9-15.
- PROSSER, C. L., & F. A. BROWN, JR.
1961. Comparative animal physiology, 2nd edition. Saunders, Philadelphia. x + 688 pp.
- RAND, A. L.
1942. Results of the Archbold Expeditions. No. 44. Some notes on bird behavior. Bull. Amer. Mus. Nat. Hist., 79: 517-524.
- REMOLD, H.
1963. Scent-glands of land-bugs, their physiology and biological function. Nature, 198: 764-768.
- RICHTER, C. P.
1957. Hunger and appetite. In Symposium on nutrition and behavior, ed. Josef Brozek, Nutrition symposium series No. 14. National Vitamin Foundation, N. Y. p. 39.
- ROBINSON, T.
1963. The organic constituents of higher plants, their chemistry and interrelationships. Burgess Publishing Co., Minneapolis, Minn. 306 pp.
- ROTH, L. M., & T. EISNER
1962. Chemical defenses of arthropods. Ann. Review of Entomology, 7: 107-136.
- ROTH, L. M., W. D. NIEGISH & W. H. STAHL
1956. Occurrence of 2-hexenal in the cockroach *Eurycotis floridana*. Science, 123: 670-671.
- ROTHSCHILD, MIRIAM
1964. An extension of Dr. Lincoln Brower's theory on bird predation and food specificity, together with some observations on bird memory in relation to aposomatic colour patterns. Entomologist, 97: 73-78.
- ROTHSCHILD, MIRIAM, & J. PARSONS
1962. Pharmacology of the poison gland of the Locust, *Poeciloceris bufonius* Klug. Proc. Roy. Ent. Soc. London, Series C, 27: 21-22.
- SATO, M.
1963. The effect of temperature change on the response of taste receptors. In Olfaction and taste, ed. Y. Zotterman, Pergamon Press, London. pp. 151-164.

- SEITZ, A.
1924. Editor, The Macrolepidoptera of the World. Vol. 5: The American Rhopalocera. Alfred Kernen Verlag, Stuttgart. 1139 pp., 203 col. pls.
- SEVASTOPULO, D. G.
1952. Aposematic butterflies protected by the poisonous qualities of their larval food-plants. Jour. Bombay Nat. Hist. Soc., 50: 951-952.
- SHEPPARD, P. M.
1963. The genetics of mimicry. Symposium on mimicry. Proc. XVI International Congress of Zoology, Washington, D. C. Vol. 4: 150-156.
- SLATER, J. W.
1877. On the food of gaily-coloured caterpillars. Trans. Ent. Soc. London, 1877: 205-209.
- SMITH, J. N.
1955. Detoxication mechanisms in insects. Biological Reviews, 30: 455-475.
- SNEDECOR, G. W.
1946. Statistical methods. 4th edition. Iowa State College Press, Ames, Iowa. 485 pp.
- STEYN, D. G.
1962. Grasshopper (*Phymateus leprosus* Fabr.) poisoning in a Bantu child. South African Medical Journal, 36: 822-823.
- STURTEVANT, E. L.
1919. In Sturtevant's notes on edible plants. Ed. by U. P. Hedrick. Report N. Y. Agricultural Expt. Station for 1919. Albany, J. B. Lyon & Co. 686 pp.
- SWYNNERTON, C. F. M.
1915. Concluding discussion. In G. D. H. Carpenter (1942). Observations and experiments in Africa by the late C. F. M. Swynnerton on wild birds eating butterflies and the preference shown. Proc. Linn. Soc. London, 154: 10-46.
1919. Experiments and observations bearing on the explanation of form and colouring, 1908-1913, Africa. Jour. Linnaean Soc., Zoology, 33: 203-385.
- TEITELBAUM, P., & A. N. EPSTEIN
1963. The role of taste and smell in the regulation of food and water uptake. In Olfaction and taste, ed. Y. Zotterman. Pergamon Press, London. pp. 347-380.
- TSCHESCHE, R., D. FORSTMANN & V. K. M. RAO
1958. Zur Kenntnis der Cardenolid-Inhaltsstoffe von *Asclepias curassavica* L. Chemische Berichte, 91: 1204-1211.
- TSCHESCHE, R., G. SNATZKE & G. GRIMMER
1959. Calotropagenin aus *Asclepias curassavica* L. Die Naturwissenschaften, 46: 263-264.
- WALLACE, A. R.
1867a. No title. Proc. Ent. Soc. London, 1867: 80-81.
1867b. On the Pieridae of the Indian and Australian regions. Trans. Ent. Soc. London, 1867: 301-416.
1869. No title. Proc. Ent. Soc. London, 1869: vii.
1870. Contributions to the theory of natural selection. Macmillan & Co., London. Chapter 3, pp. 45-129.
1889. Darwinism. Macmillan and Co., London. xvi + 494 pp.
- WATERHOUSE, D. F., D. A. FORSS & R. H. HACKMAN
1961. Characteristic odour components of the scent of stink bugs. Jour. Insect Physiology, 6: 113-121.
- WATT, J. M., & MARIA G. BREYER-BRANDWIJK
1932. The medicinal and poisonous plants of Southern Africa. E. and S. Livingstone, Edinburgh. 314 pp.
- WEBB, L. J.
1955. A preliminary phytochemical survey of Papua-New Guinea. Pacific Science, 9: 430-441.
- WEIR, J. JENNER
1869. On insects and insectivorous birds; and especially on the relation between the colour and the edibility of Lepidoptera and their larvae. Trans. Ent. Soc. London, 1869: 21-26.
1870. Further observations on the relation between the colour and the edibility of Lepidoptera and their larvae. Trans. Ent. Soc. London, 1870: 337-339.
- WEISMANN, A.
1882. Studies in the theory of descent. Translated by R. Meldola. Sampson Low, Marston, Searle, and Rivington, London. 729 pp.
- WILLAMAN, J. J., & BERNICE G. SCHUBERT
1961. Alkaloid-bearing plants and their contained alkaloids. Government Printing Office. U. S. Dept. Agriculture Tech. Bull. No. 1234, 287 pp.
- WILLIAMS, R. O., & E. E. CHEESMAN
1947. Flora of Trinidad and Tobago. Vol. II, part 3; pp. 123-187. Guardian Commercial Printery, Port-of-Spain, Trinidad.
- WILLIAMS, R. TECWYN
1959. Detoxication Mechanisms. Chapman & Hall Ltd., London. 796 pp.
- WOODSON, R. E., JR., H. W. YOUNGKEN, E. SCHLITTER & J. A. SCHNEIDER
1957. Rauwolfia. Botany, Pharmacognosy, Chemistry and Pharmacology. Little, Brown & Co., Boston. 149 pp.
- WYNTER-BLYTH, M. A.
1957. Butterflies of the Indian Region. Bombay Nat. Hist. Soc., Bombay. 523 pp.